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Characterization of thermal, mechanical and hydration properties of novel films based on *Saccharomyces cerevisiae* biomass

Juan Francisco Delgado[1,2*], Mercedes A. Peltzer[1,2], Andrés G. Salvay[1], Orlando de la Osa[1] and Jorge R. Wagner[1,2].

[1]Department of Science and Technology, National University of Quilmes, Roque Sáenz Peña 352, (B1876BXD) Bernal, Buenos Aires, Argentina.

[2]National Scientific and Technical Research Council (CONICET), Godoy Cruz 2290 (C1425FQB), Ciudad Autónoma de Buenos Aires (CABA), Argentina.

*Address correspondence to:

Juan F. Delgado, Department of Science and Technology, National University of Quilmes, Roque Sáenz Peña 352, (B1876BXD) Bernal, Buenos Aires, Argentina; E-mail: juan.delgado@unq.edu.ar

Abstract

Characterisation of films made of *Saccharomyces cerevisiae* biomass was performed so as to better understand their properties. The treated yeast biomass, named HTH, was prepared by subsequent steps including a first homogenisation, heat treatment and final homogenisation. Glycerol was added as plasticizer and films were evaluated using different techniques to measure mechanical behaviour, thermal properties and water sorption. Temperature of maximum decomposition rate was found near 300 °C, while the gradually disappearance of amide II band (1543 cm^{-1}) occurred from 225 °C, identified by FTIR spectra of residues of

thermally treated films by TGA. A glass transition at 64.5±3.0 °C and two low energy endotherms at 61.2±2.3 and 106.1±0.7 °C were determined in completely dehydrated films without glycerol. When glycerol content was increased the glass transition temperature was shifted to lower values, a maximum elongation of 12 % was reached, Young's modulus decreased from 88 to 9 MPa and the water uptake of films raised from 0.49 to 0.79 g H_2O/g of dry matter. The characterisation of yeast biodegradable films provided evidence of their properties for a potential future use in packaging industry.

Keywords: biodegradable films, yeast, characterization, modulated-temperature differential scanning calorimetry, infrared spectroscopy, tensile tests, water sorption.

1. Introduction

Research and developments on biodegradable materials are hot topics in materials science (Atarés & Chiralt, 2016; Azeredo & Waldron, 2016; Babu, O'Connor, & Seeram, 2013; Han, 2013; Siracusa, Rocculi, Romani, & Rosa, 2008; Sudesh, & Iwata, 2008). At least two reasons are behind this trend: the non-renewable character of materials obtained from petroleum and the pollution generated in our ecosystem from misuse and wrong disposal of objects made from synthetic non-biodegradable polymers. In last decades, an alternative proposed for many authors was the use of biodegradable polymers (Mahalik & Nambiar, 2010; Tharanathan, 2003) obtained from renewable and sustainable sources for some packaging applications. There are many different origins of these biopolymers, in the case of polyhydroxyalkanoates (PHA), such as polyhydroxybutirate or valerate (PHB-PHV) (Suriyamongkol, Weselake, Narine, Moloney, & Shah, 2007), are completely synthesized from bacteria, while polylactic acid (PLA) is obtained from glucose fermentation and further polymerization reactions of lactic acid. PLA is the most extended biodegradable polymer due to its excellent mechanical properties, transparency and low interaction with water molecules (Jiménez, Peltzer, & Ruseckaite, 2014). On the other hand, macromolecules from agroindustrial sources have been studied intensively (Burgos, Valdés, & Jiménez, 2016). This group includes polysaccharides: amylose and

amylopectin from various types of starch (García, Martino, & Zaritzky, 2000; Mali, Grossmann, García, Martino, & Zaritzky, 2006), cellulose (Orelma, Filpponen, Johansson, Laine, & Rojas, 2011), chitosan (Chatelet, Damour, & Domard, 2001; P. K. Dutta, Tripathi, Mehrotra, & J. Dutta, 2009), pectins; proteins: casein and milk whey proteins (Kurek, Galus, & Debeaufort, 2014), soy protein isolate (Kokoszka, Debeaufort, Hambleton, Lenart, & Voilley, 2010), gelatine (Nur Hanani, O'Mahony, Roos, Oliveira, & Kerry, 2014), corn zein (Kashiri et al., 2016) and others. Lipids are not suitable to form matrices with acceptable mechanical properties, but they are utilized to reduce the strong interaction with water of films and coatings made of hydrophilic biopolymers and intended to be used as food packaging.

Biopolymers may also proceed from industrial by-products like milk whey, crustacean exoskeletons, husks from beans or grains, etc. (Burgos et al., 2016). In this way, the use of these sources makes possible the production concept of eco-efficient production 'cradle to cradle' (Braungart, McDonough, & Bollinger, 2007; McDonough & Braungart, 2002) where the waste from one process may be the input of another process. Many authors have reported physical properties of films prepared from a wide variety of agroindustrial biopolymers. Regarding yeast cells, brewer's spent yeast biomass could be a reliable source to obtain the raw material to develop films and packages (Ferreira, Pinho, Vieira, & Tavarela, 2010, Delgado, et al, 2016). Yeast biomass is a viable source of biopolymers such as proteins and polysaccharides for producing biodegradable films as described by Delgado et al., 2016. Composition of pressed bakers' yeast was determined by the authors being RNA (%): 7.72 ± 0.04; proteins (%): 41.2 ± 0.4 and carbohydrates (%): 46.6 ± 1.2. Also, the effect of different pressures applied on homogenisation on water retention capacity and dispersible matter quantity was studied. Authors used a combination of homogenisation, heat treatment and a further homogenisation to produce films with good attributes, great continuity and homogeneity without punctures. After casting films presented a characteristic amber colour. However, a complete characterisation is necessary to propose better applications to these films or to perform modifications to them, such

as plasticization. There is no previous background of the use of total yeast biomass to make films, although some components of yeast cell such as β-glucans from cell wall were used to obtain films (Novák et al., 2012) or to encapsulate active compounds (Shi et al., 2007). Advantages of using the full biomass of yeast are the reduction of the dismissal of valuable components and the decrease of purification steps of selected components that increase the obtaining cost.

In order to take advantage of yeast components, it is necessary to break cell wall and release cytoplasmatic content. There are many methods for cell disruption (Liu, Ding, Sun, Boussetta, & Vorobiev, 2016) but, high pressure homogenisation is the best choice to scale-up the production. It is necessary pressures higher than 60 MPa to considerably damage cell walls and begin a massive release of the cytoplasm (Spiden, Scales, Kentish, & Martin, 2013). Thereby, polymers are completely free to interact and form a stable network. A group of treatments should be applied on yeast biomass in a certain order to obtain dispersions capable to form films. The first homogenisation step is intended to break up cell wall and release cytoplasm and thermal treatment denatures proteins and increases interactions between them. Then, the second homogenisation disassembles large aggregates. This sequence of combined treatments was named as HTH, and exhibited the best performance to obtain films in a previous work (Delgado et al., 2016).

Plasticizers increase film flexibility due to their ability to reduce internal hydrogen bonding between polymer chains while increasing molecular space. The most effective plasticizers will generally resemble most closely the structure of the polymer that they plasticize, thus, the most commonly used plasticizer in biopolymers based films are polyols, such as glycerol (Mali, Grossmann, García, Martino, & Zaritzky, 2004). Films made of natural biopolymers are brittle and have poor elongation capacity, either for their industrial processing or final application it is necessary the incorporation of plasticizers to improve the handleability and processability. The main objective of this study was to characterise films obtained from yeast biomass in three

central aspects: mechanical, hydration and thermal behaviour and their relationship with glycerol as plasticizer.

2. Materials and methods

2.1 Preparation of films

As described previously, a dispersion of 10 % w (dry matter of yeasts)/volume of dispersion (Commercial pressed bakers' yeast, Calsa, AB Mauri, Tucumán, Argentina) was prepared using distilled water (Delgado et al., 2016). Before use, yeasts cells were cleaned dispersing them in distilled water and centrifuging at 1,000 g. Supernatant was wasted and sedimented cells were used to prepare the initial dispersion.

The 10 % w/v dispersion was submitted to high pressure homogenisation at 125 MPa (H), and continuous flow during 9 min (High pressure homogeniser NS1001L, Niro Soavi, Italy), followed by a thermal treatment (T) in a water bath at 90 ºC for a time lapse of 20 min. A second high pressure homogenisation (H) step was applied under the same conditions as the first homogenisation process, and treated yeast biomass dispersion namely as HTH was obtained. Final pH of dispersions was in the range of 5.8-5.9. Glycerol was added to dispersion at levels of 10, 20 and 30 % w/w respect to dry matter (HTH-10Gly, HTH-20Gly and HTH-30Gly, respectively). Dispersions without glycerol were prepared as a control (HTH-0Gly). Twenty millilitres of each dispersion were discharged in plastic Petri dishes (90 mm of diameter) and water evaporation was done at 50 °C in a ventilated oven, until the remaining water content of the films was between 10-15 %. In figure 1, a simplified scheme of the film obtaining process can be seen. Samples obtained were stored at 43 % relative humidity (r.h.) or other conditions specified in each study.

FIGURE 1

2.2 Modulated-Temperature Differential Scanning Calorimetry (MDSC)

In order to understand the thermal behaviour of HTH films and how they were affected by the presence of glycerol, samples with different percentages of plasticizer were conditioned at 21 ºC and 0 % r.h. using silica gel until reach equilibrium in desiccators. Determination of relevant thermal events in dried yeast films and the effect of plasticizer over them were evaluated.

Approximately 7 mg of sample were placed into Tzero aluminium pans and sealed with hermetic lids. Studies were conducted using a Modulated-Temperature Differential Scanning Calorimeter (TA Instruments Q200, Delaware, USA), in the range of -80 ºC to 150 ºC with a previous equilibration step at -80 ºC during 5 minutes, and then temperature was increased at 10 ºC min^{-1} with ±1 °C of modulation amplitude and 40 seconds of time period. Thermograms were analysed from its reversible and non-reversible heat flow. Analyses were performed in triplicates and glass transitions were determined in reversible heat flow using TA Universal Analysis software (v4.5, TA Instruments, USA) at the mid-point. Meanwhile, peak temperature and the enthalpy associate to endotherms were determined in the Non-Reversible Heat Flow signal.

2.3 Thermogravimetric analyses (TGA)

Mass loss in samples as function of temperature was registered by TGA in a Q-500 thermogravimetric analyser (TA Instruments, Delaware, USA). Approximately 10 mg of sample were weighed and analyzed by heating at 20 ºC min^{-1} under inert nitrogen atmosphere (flow rates: 40 mL min^{-1} in balance and 60 mL min^{-1} in sample), from 50 °C to 600 °C. From TGA curves, initial degradation temperatures (T_{90}) were calculated and maximum degradation rate and temperature of each degradation zone (T_{peak}) were obtained from the derivative of weight with respect to temperature. Initial degradation temperature was considered at 10 % of weight loss (T_{90}) because it was necessary to compare samples without the influence of water. Determinations were performed in triplicates. Additionally, a sample of pure glycerol was submitted to the same procedure with the objective to know relevant thermal decomposition events of this compound and relate them with the behaviour of plasticized film samples.

In order to study the thermal degradation of films, residues of samples without plasticizer were obtained at different temperatures. Approximately 30 mg of samples were weighed and subjected to heating at 10 ºC min^{-1} from 50 ºC to different final temperatures: 120, 150, 180, 200, 225, 250 and 275 ºC. These residues were analyzed by infrared spectroscopy (ATR-FTIR) in duplicates.

2.4 Attenuated Total Reflectance-Fourier Transform Infrared analyses (ATR-FTIR)

Infrared spectra of films and residues described in section 2.3 were registered in the range of 4000 to 400 cm^{-1} on a Fourier-Transform Infrared Analyzer (FTIR) Shimadzu IR-Affinity (Shimadzu Co., Japan) equipped with an attenuated total reflectance diamond module (GladiATR, Pike Technologies, USA). IR spectra were measured as an average of 45 scans, a resolution of 4.0 cm^{-1} and Happ-Genzel appodization.

2.5 Mechanical properties

Uniaxial tensile tests were done in a universal testing machine, Megatest® TC-500 II Series (Micrometric SRL, Buenos Aires, Argentina). Films were cut according to ASTM D1708-00, further information about dimensions can be found in Appendix A: supplementary data ~~and dimensions of the obtained specimens are showed in Figure 2~~. Temperature was controlled at 21 ºC and specimens of HTH films with different amounts of plasticizer (10, 20 and 30 % w/w d.m.) were conditioned to 65 % of r.h. through a $NaNO_2$ saturated solution. Samples with no plasticizer were not assayed due to their high brittleness that makes difficult the measurements. The average thickness of specimens was 0.30±0.03 mm as a result of six independent measurements in each specimen, measured by a digital calliper. The selected speed to perform tests was 5 mm min^{-1} and at least five specimens of each composition were tested. A 30 N load cell was used and the initial grip separation was 22 mm. Deformation at break (%), Young's modulus (calculated in the initial linear region) and ultimate tensile strength were determined.

2.6 Water sorption isotherms

Sorption isotherms of films were determined gravimetrically according to standard procedure (Bertuzzi, Castro Vidaurre, Armada, & Gottifredi, 2007). Samples of fully dried films with different percentages of glycerol and a superficial area of approximately 58 cm^2 were placed in containers of 1.5 litre and equilibrated at different relative humidities (r.h.) at 24 ºC. For this, saturated solutions of LiCl, $MgCl_2$, K_2CO_3, NaBr, NaCl, KCl, and $BaCl_2$ were used to generate a_w of 0.11, 0.33, 0.43, 0.57, 0.75, 0.84, and 0.90, as informed previously by Spiess & Wolf (1983). Silica gel was used to generate an atmosphere of approximately 0 % r.h. The evolution to equilibrium at each moisture conditions was monitored by weighting periodically the samples until constant weight. Samples were weighed using an analytical balance ($\pm 10^{-4}$ g). The water content or hydration *h*, given in units of g of water per g of dry matter (d.m.) was evaluated as function of a_w. Experiments were performed at 24 ºC and repeated two times.

Isotherms were fitted using Guggenheim-Anderson-DeBoer (GAB) model (Guggenheim, 1966) through the Eq. 1:

$$h\ (a_w) = \frac{N.c.k.a_w}{[(1+(c-1)k.a_w)(1-ka_w)]} \quad (1)$$

Latex code: h(a_{w})=\frac{N.c.k.a_{w}}{[(1+(c-1)k.a_{w})(1-ka_{w})]}

where *N* is the monolayer water content (g of water per g of dried mass) related to the number of primary binding sites of water molecules, *c* is a parameter related to the difference between the chemical potential of the water molecules in the upper layers and in the monolayer, and *k* is a factor related to the difference between the chemical potential of the water in pure liquid state and in the upper layers (Guggenheim, 1966). Parameter *c* can also be interpreted as a quantity related to the force of the water binding to monolayer, and *k* is a parameter that denotes the capability of water to be bound to the multilayer (Salvay et al., 2003).

2.7 Kinetics of water sorption

Kinetics of water vapour sorption was measured at 24 °C. Fully dried film samples were placed into the sorption containers at 90 % r.h. The samples were removed at specific intervals of time and weighed for data collection and experiments were repeated two times.

The water content h in g H_2O per g d.m. as function of time t was fitted using the Pilosof-Boquet-Bartholomai (PBB) model (Pilosof, Boquet, & Bartholomai, 1985), through the Eq. 2:

$$h(t) = h_0 + \frac{h_\infty t}{B + t} \qquad (2)$$

Where h_0 is the initial water content, h_∞ the final water content at equilibrium, and B the time (day) when $h(t)=h_\infty/2$. Through Eq. 2, the rate kinetic constant $K=1/(h_\infty B)$ can be obtained in units of g of dried mass per g of sorbed H_2O per day.

2.8 Statistical analysis

PSPP 0.8.5 (Free Software Foundation, Boston, USA) and R 3.2.2 (The R Foundation for Statistical Software, Vienna, Austria) were used to analyze data. Analysis of Variance (ANOVA) and multiple comparisons test (HSD Tukey) were used at a confidence level $p<0.05$.

3. Results & discussion

3.1 Thermal characterisation of films

3.1.1 Modulated-Temperature Differential Scanning Calorimetry

HTH films without addition of glycerol were very brittle at room conditions; this is due to the glassy state of the samples as T_g was over room temperature as was encountered in MDSC thermograms (Figure 2). Samples were studied in dry conditions in order to observe thermal events independently to the films water content that could also plasticize the matrix. When plasticizer was added to formulations T_g tended to decrease, as expected (Figure 2). Sample HTH-0Gly presented the T_g at 64.5±3.0 ºC and was shifted to 12.5±4.2 ºC when 10% of glycerol was added, see HTH-10Gly. Further addition of glycerol produced a decrease in T_g values and T_gs were encountered at 4.7±1.2 ºC and -0.3 ±1.2ºC, for samples HTH-20Gly and HTH-30Gly, respectively. Unlike what happens in amorphous samples of pure substances or simple composition formulations, where the glass transition temperature involves a narrow temperature range, multi-component samples composed by proteins and polysaccharides, may involve larger temperature ranges and its shape may not be as sharp as in synthetic polymers (Jara & Pilosof, 2009). In non-reversible heat flow two endotherms were observed when no glycerol was added, temperatures of these endotherms were 61.2±2.3 ºC and 106.1±1.0 ºC. It is possible that these events are related to β-glucans (Novak et al., 2012). The first endotherm was present in all formulations (plasticized and non-plasticized) and its associated energy ranged from 3.4±0.9 J/g in HTH-0Gly to 3.1±1.1 J/g in HTH-30-Gly seemed to be unchanged, but the second endotherm was very reduced and was not present when the plasticizer was incorporated as shown in Figure 2. MDSC technique allowed to recognize overlapped events in HTH-0Gly (glass transition and endotherm), remarking the utility of this capability.

FIGURE 2

In polymeric matrices the plasticizer molecules are believed to reduce the attractive force between polymer chains in the low moisture matrix film, to increase the flexibility of the system (Lieberman & Gilbert, 1973). It is important to note that the hydration water of a hydrophilic film also acts as a plasticizer and films become more fragile in low hydration conditions (Coupland, Shaw, Monahan, Dolores O'Riordan, & O'Sullivan, 2000). The study of changes in

the glass transition (T_g) to explain the effect of plasticizer molecules on the behaviour of films was proposed previously by many authors (Kristo et al., 2010; Mali, Grossmann, García, Martino, & Zaritzky, 2006; Gontard & Ring, 1996). It is important to know the temperature at which the change between the glassy and rubber state occurs to propose a correct application to the material, as well as to know the compatibility between the plasticizer and the polymer or mixture of polymers used and the effect of the amount of plasticizer added. If a very large or little amount of plasticizer is used, the moisture barrier and/or mechanical properties of the film fail with respect to the selected application (McHugh & Krochta, 1994).

3.1.2 Thermogravimetric analysis

Thermogravimetric analyses of samples showed a multiple degradation profile with different degradation zones as shown in Figure 3, where weight (%) and derivative of weight (% $^oC^{-1}$) were described versus temperature. First degradation zone, up to 140 ºC was attributed to water evaporation and low-molecular-weight molecules degradation. The second zone, from 140 °C to 225 °C, possibly implies the beginning of the partial degradation of the protein, as it will be proven with FTIR assays in the section 3.2. Similar results were obtained by Guerrero, Retegi, Gabilondo, & de la Caba (2010) in films made from soy protein isolates and they reported substantial degradation of the material above 180 °C. In this second zone, there are at least two different events, the first one with the T_{peak} around 175 ºC and the second one at 211 ºC. These temperatures tended to decrease with the addition of plasticizer at different levels but there were no statistically significant differences. On the other hand, the intensity and magnitude of the second maximum rate was affected by the presence of glycerol showing the influence of the plasticizer in this transition zone. An additional test (data not shown) was performed to pure glycerol and the TGA profile showed the initial degradation temperature at 152 ºC and the T_{Peak} at 247 ºC, these data were consistent with those described in the literature (Arrieta, Peltzer, Garrigós & Jiménez 2013). Therefore, the degradation of the glycerol started in the second

degradation zone and continues in the third zone. The third degradation zone from 224 ºC to 359 ºC, with the T_{peak} nearly 309 °C was attributed to the pyrolysis of β-glucan and to the massive decomposition of proteins and other organic compounds (Novák et al., 2012; Roca et al., 2012; Rudnik, Matuschek, Milanov, & Kettrup, 2006; Wu et al., 2013). Finally, after 359 ºC starts the last zone, which presents two more events that complete the thermal degradation of samples. Final residue at 600 ºC was logically affected by the presence and quantity of glycerol added. Carbon residues decreased from 28.1±1.0 % to 18.6±0.9 % for samples with 0 % and 30 % of glycerol respectively. Table 1 shows the parameters obtained from TGA curves such as T_{90} and T_{Peak} corresponding to 2nd and 3rd degradation zones and the final residues at 600 ºC. T_{90} was affected by the presence of glycerol and drop in 50 ºC for plasticized samples HTH-20Gly or HTH-30Gly. There is an effect due to a plasticization of the matrix that exposes polymer chains even more to the thermal degradation.

In the light of these results, the processing of plasticized yeast material could be carried out up to 150 ºC without any substantial degradation in the polymer matrix. Others authors have reported lower process temperatures than 150 ºC to obtain blow-films from sodium caseinate using a co-rotating twin-screw extruder (Belyamani, Prochazka, & Assezat, 2014).

FIGURE 3

TABLE 1

3.2 Attenuated Total Reflectance-Fourier Transform Infrared analyses (ATR-FTIR)

In Figure 4, the spectra of HTH films formulations and glycerol are showed and typical bands of glycerol and *Saccharomyces cerevisiae* are well described and correspond to the already reported spectra for these specimens (Burattini et al., 2008; Padmavathy, Vasudevan, & Dhingra, 2003; Galichet, Sockalingum, Belarbi, & Manfait, 2001; Šandula, Kogan, Kačuráková, & Machová, 1999). The spectra showed important peaks related to proteins like amide bands, nucleic acids and to glucans. The spectrum of a macromolecule with a great variety of

functional groups or mixes of components could have a complex interpretation and the attribution of each band could be a difficult job. Padmavathy et al. (2003) divided in four zones the spectrum of the native yeast. These authors identified a first region related with –OH and –NH functional groups (3800-2800 cm^{-1}), a next zone related to proteins, that includes amide I, II, and others (1650-1400 cm^{-1}), a third narrow zone (1290-1200 cm^{-1}) corresponding to nucleic acids and a fourth zone (1180-790 cm^{-1}) of sugars. Regarding HTH films, independently of the glycerol content, the band observed at 3271 cm^{-1} is due to the flexion of hydroxyl groups, and bands in 2922 cm^{-1} and 2852 cm^{-1} corresponds to vibrations in α carbon directly bonded to hydroxyl. In protein region, four bands stand out: 1622 cm^{-1}, 1541 cm^{-1}, 1456 cm^{-1} and 1398 cm^{-1}. Amide I (1622 cm^{-1}) is due to the stretching of the C=O bond, while amide II has a major contribution of N-H bending in the amide bond and a minor contribution of the C-N bending. The absorption band at 1230 cm^{-1} was described by many authors (Burattini et al., 2008; Padmavathy et al., 2003; Galichet et al. 2001) and was attributed to the asymmetric stretching of PO_2^- from nucleic acids and phospholipids. The presence of phospholipids was also evidenced by the absorption band at 1750 cm^{-1}. Numerous bands next to 1000 cm^{-1} are related to the presence of pyranose, glucans or mannans (920 cm^{-1}, 995 cm^{-1}, 1031 cm^{-1}, 1103 cm^{-1}, 1147 cm^{-1}) (Salman et. al, 2010, Šandula et al., 1999). The strong and broad peak observed at 1031 cm^{-1}corresponds to the contributions of mannans and glucan (Burattini et al., 2008). Many authors have informed a peak at 1080 cm^{-1} related to the symmetric stretching in phosphate groups in nucleic acids (Salman et al., 2010). However, the intensity of this peak is probably overlapped with the peak at 1076 cm^{-1} due to polysaccharides (β-glucan).

FIGURE 4

Glycerol influences several peaks in yeast biomass films. In Figure 4, spectra obtained from samples with and without glycerol added are shown, glycerol spectrum is provided as a comparison. As expected, the bands at 3265 cm^{-1}, 2877 cm^{-1}, 1405 cm^{-1}, 1230 cm^{-1}, 1107 cm^{-1}, 1031 cm^{-1}, 986 cm^{-1}, 921 cm^{-1} and 850 cm^{-1} increased its intensity when higher amounts of

glycerol were added, because the plasticizer has a significant absorbance at these wavenumbers. In the case of 1030 cm^{-1} peak, the increase when glycerol was added was notorious and a little shift to lower wavenumbers of the spectrum was produced, whereas amide II band (1456 cm^{-1}) decrease its intensity; this behaviour could be attributed to the sensitivity to hydrogen bonding of this functional group (Gao et al., 2006).

The evaluation of the HTH-0Gly film residues obtained after heating up to different temperatures was conducted by ATR-FTIR, in order to understand how macromolecules that constitute the yeast film are degraded during the TGA assay.

No appreciable changes were observed in those residues obtained after heating the sample up to 120, 150, 180 and 200 ºC (Figure 5). This means that when heating the samples up to 200 ºC, the degradation events occurred in TGA may correspond to partial protein degradation that involves side-chain residues and not the breakdown of the peptide union (Kasarda & Black, 1968). However, from 225 ºC to 275 ºC several changes were observed in ATR-FTIR spectra, such as the disappearance of the amide II band. At the same time, the band corresponding to polysaccharides at 1031 cm^{-1} increased its intensity at temperatures from 225 ºC to 250 ºC, and a clear separation of bands at 1031 and 1076 cm^{-1} was observed. Since mannans are linked to proteins (forming mannoproteins) and degradation of polysaccharides begins to be incipient at 250 ºC, changes observed in spectra are reasonable.

FIGURE 5

These results are in accordance with previous results obtained from other biodegradable materials like milk proteins and gelatine (Barreto, Pires, & Soldi, 2003). In those studies, it was informed that over 200 ºC an important thermal degradation of amide took place, decreasing the relative intensity between amide I and II. Due to amide II band degradation implies ammonia releasing from the sample, the same authors observed the ammonia absorption bands at 930, 970 and 3335 cm^{-1} in residual gases from TGA conducted to FTIR.

3.3 Mechanical properties

The addition of plasticizer resulted in a substantial reduction on the Young's modulus and maximum strength values, as showed in Table 2. This result clearly exemplified plasticizer work at the molecular level as produced changes in the spacing between molecules, decreased protein - protein or glucan - glucan and protein – glucan interactions, allowing greater mobility. This increased capability of movement, resulted macroscopically in rubber like characteristic state. The increase in the amount of plasticizer, decreased interaction between chains as mentioned, the mobility was increased though rigidity and brittleness of films decreased. The significant decrease in the Young's modulus with plasticization was not accompanied by concomitant increase in the deformation at break value as expected.

The values obtained in the Young's modulus and the maximum strength were similar to those reported in films based on soy protein isolate with significantly higher amounts of glycerol (Guerrero et al., 2010). In this work the authors also observed a decrease in tensile strength and Young's modulus with the addition of plasticizer. In contrast to our work, soy protein films had a better performance regarding deformation at break.

TABLE 2

Ciannamea et al. (2014) also informed that in soy protein concentrate films with the addition of high amounts of glycerol (30 – 50 %) the elongation at break was around 15-20 %. Then, Novák et al. (2012) reported films produced with purified yeast β-glucan and 25 % w/w of glycerol with a Young's Modulus several times higher (712±31 MPa) than the modulus showed in this work when tensile assays were developed at 43 % r.h. However, similar deformation at break was observed (14.16±1.11 %) for the purified biopolymer in comparison to whole yeast biomass films.

3.4 Hydration properties

A key role of packaging materials including edible films is to reduce the exchange of water between the food and the environment, in particular to reduce drying of moist foods. The barrier properties of a film to water vapour depend on both the molecular diffusion coefficient and the solubility of water in the film (McHugh & Krochta, 1994). The kinetic of water sorption allows obtaining information about the mobility of the water in the film through determination of the rate of hydration and the water sorption isotherms are a way to characterize the solubility of water in films.

3.4.1 Kinetics of water sorption

Figure 6A displays plots representing the kinetics of water sorption at 90 % r.h. for films with percentages of glycerol of 0, 10, 20, 30 % w/w d.m. Table 3 shows parameters obtained from the fittings of experimental data with Eq. 2. It was found that the addition of glycerol in the films produce an increment in h_{∞}, the hydration in equilibrium at 90 % r.h., and a decrease in the rate kinetic constant K. It was suggested that the addition of glycerol reduces intermolecular forces between polymers chains in the film, spacing them (Jangchud & Chinnan, 1999). This effect appears to be increasing the number of water molecules hydrating the film. On the other hand, the rate which the hydration process reaches the equilibrium decreases when glycerol content increases. This may be due to the fact that a greater number of water molecules must be accommodated in the polymeric matrix, increasing the time needed to reach equilibrium.

FIGURE 6

3.4.2 Water sorption isotherms

Sorption isotherms of the films at percentages of glycerol of 0, 10, 20, 30 % w/w d. m. are shown in Figure 6B. Table 4 shows parameters obtained from fitting the experimental data with Eq. 1. It is possible to see in Figure 6B that the incorporation of glycerol in samples produced

an increase in the amount of hydration water without any modification on the shape of the isotherms. All sorption isotherm curves can be viewed as two distinct regions indicating that the equilibrium moisture increases slowly with an increase in water activity up to 0.6, beyond which there was a sharp increase in equilibrium moisture of the films studied. This shape of sorption isotherms suggests a hydration mainly in forms of multilayer, with a small monolayer of hydration. In this way, the water hydration is mobile water that is not strongly bounded to the film. Results from Table 4 show that the parameter *N* is not altered by the presence of glycerol, while *c* and *k* increase with the amount of glycerol in the film.

The shape and the amount of water content in the isotherm of the film without glycerol of Figure 6B was similar to other films without plasticizer as cellulose-based edible films (Chinnan & Park, 1995), starch-based films (Bertuzzi et al., 2007), hydroxypropilmethylcellullose based films (Pastor Navarro, 2010), myofibrillar protein-based films (B. Cuq, Gontard, J. L. Cuq, & Guilbert, 1997), and sodium caseinate based films (Kristo, Koutsoumanis, & Biliaderis, 2008).

Glycerol increased the amount of hydration water without changes on the shape of the isotherms showed in Figure 6B and it was also observed from cassava starch film (Mali et al., 2006) and whey protein films (Coupland et al., 2000). However, in these films the GAB parameter N increased and *c* decreased with increased glycerol concentration, whilst *k* was independent of glycerol content. The parameter *k* initially increased when glycerol was added to cassava starch film and from there was independent of glycerol content (Mali et al., 2006).

TABLE 4

Our results suggest that in yeasts-based film the presence of glycerol increase the force of water binding to monolayer without alter the number of primary binding sites, and augmenting the capability to water binding to multilayer.

According to the results obtained in the hydration experiments, as in the mechanical and thermal degradation tests, this material could be processed industrially at temperatures below 120 ºC and

used to protect fatty and/or low water food products such as dried fruits as well as in non-food applications. Indeed, some application may concern their use in temporary protection of low hygroscopic powders.

4. Conclusions

Biopolymers from agro-industrial sources greatly interact with water and their mechanical and water vapour barrier properties are far from those corresponding to synthetic polymers. Some modifications are necessary to do in order to improve their performance and apply them as food packaging.

Since the applicability or potentiality of these novel films based on yeast biomass films depends on their properties, a complete characterisation covering relevant aspects as thermal, mechanical and hydration properties was carried out, analyzing how they were influenced by the inclusion of glycerol. The increasing in plasticizer content decreased Young's modulus and tensile strength, but increased slightly the elongation at break. Indeed, the presence of plasticizer affected the amount of hydration water and also had impact in thermal degradation: Therefore, the amount of plasticizer to be incorporated comprises a commitment in all of them.

At the moment yeast biomass constitutes an alternative to other sources of biopolymers and the availability of these resources, such as residual yeasts from brewing and wine industry push forward their use. This work provides a first look at the properties of yeast biomass plasticized films.

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Figure and table captions

Fig. 1. Process to obtain films from yeast biomass

~~**Fig. 2**. Dimensions of tested specimens for mechanical analysis~~

Fig. 2. Modulated-Temperature differential scanning calorimetry (MDSC) thermograms of HTH samples without plasticizer and different amounts of glycerol added, in the upper section Reversible heat flow signals are shown and at the bottom, the Non-reversible heat flow signals.

Fig. 3. Thermal degradation curves of HTH samples with different percentages of glycerol and first derivative of weight with respect to temperature (DTG) obtained by thermo-gravimetrical analysis (TGA).

Fig. 4. Fourier Transform-Infrared (FT-IR) spectrum of HTH films and the influence of the amount of glycerol added. Arrows mark main bands in HTH spectra.

Fig. 5. Fourier Transform-Infrared (FT-IR) spectra of samples without glycerol (HTH-0Gly) thermally degraded ~~by TGA~~ up to the indicated temperatures.

Fig. 6. Hydration curves for yeast film with different glycerol content. ***A***) Kinetics of water sorption at 90 % r.h. Experimental data was fitted with Eq. 1. ***B***) Water sorption isotherms. Experimental data was fitted with Eq. 2.

Table 1. Parameters obtained from thermal degradation assays of HTH films in the range of 30-600 ºC. Degradation zones correspond to the indicated in Figure 3. Different letters in each column indicate a statistical significant difference ($P<0.05$).

Table 2. Mechanical properties of plasticized films. Different letters in each column indicate a statistical significant difference ($P<0.05$).

Table 3. Results of hydration parameters obtained from HTH films according to Pilosof, Boquet & Bartholomai model (PBB).

Table 4.Values of the parameters fitted for the sorption isotherms displayed in Figure 6B. Isotherms were fitted with the Guggenheim-Anderson-De Boer (GAB) formula done in Eq. 1. R^2 is a statistical parameter.

Industrial relevance: *Saccharomyces cerevisiae* yeasts have many applications in food industry.

The development of biodegradable films based on yeast biomass carries many advantages such as, the possibility of using commercial pressed baker's yeast or an industrial residue from brewing industry, the use of a low-cost sources and the application of environmentally friendly procedures. The methodologies applied for the development of the film forming dispersion, high pressure homogenisation and thermal treatment, are able to be scaled-up to an industrial level.

Table 1

Sample and amount of glycerol (%)	T_{90} (ºC)	Peak Temperature				
		2nd zone			3rd zone (ºC)	Final residue at 600 ºC (%)
		$T_{peak}1$ (ºC)	$T_{peak}2$ (ºC)	T_{peak} 2 Max. Rate of decomp. (ºC min^{-1})	T_{peak} (ºC)	
HTH-0Gly	196.4±13.3[a]	168.8±11.6[a]	212.4±4.5[a]	0.09±0.02[a]	307.9±2.6[a]	27.9±0.9[a]
HTH-10Gly	208.2±15.8[a]	174.3±14.0[a]	208.8±4.4[a]	0.11±0.01[a]	306.8±6.4[a]	25.6±2.1[a]
HTH-20Gly	150.0±23.3[b]	173.8±21.9[a]	208.0±3.8[a]	0.15±0.02[b]	306.5±2.7[a]	20.6±0.5[b]
HTH-30Gly	147.5±12.2[b]	183.6±12.0[a]	215.2±8.0[a]	0.20±0.02[c]	312.8±6.2[a]	19.2±1.3[b]

Table 2

Sample	Young's Modulus (MPa)	Deformation at break (%)	Ultimate tensile strength (MPa)
HTH-10Gly	88±7[a]	7.0±1.8[a]	3.1±0.3[a]
HTH-20Gly	31±4[b]	12.1±2.1[b]	1.4±0.1[b]
HTH-30Gly	9±2[c]	11.0±1.5[b]	0.7±0.1[c]

Table 3

Sample	h_∞ (g H_2O per g d.m)	B (day)	$K=1/(h_\infty B)$ (g d.m per g H_2O per day)	R^2
HTH-0Gly	0.49 ± 0.01	1.0 ± 0.1	2.0 ± 0.2	0.9977
HTH-10Gly	0.57 ± 0.01	1.2 ± 0.1	1.5 ± 0.1	0.9978
HTH-20Gly	0.68 ± 0.02	1.5 ± 0.2	1.0 ± 0.1	0.9929
HTH-30Gly	0.79 ± 0.02	1.6 ± 0.2	0.8 ± 0.1	0.9924

Table 4

Sample	*N* (g H_2O per g d.m)	*c*	*k*	R^2
HTH-0Gly	0.08 ± 0.01	0.7 ± 0.1	0.97 ± 0.01	0.9993
HTH-10Gly	0.07 ± 0.01	0.8 ± 0.2	0.99 ± 0.01	0.9992
HTH-20Gly	0.07 ± 0.01	2.0 ± 0.7	1.01 ± 0.01	0.9982
HTH-30Gly	0.07 ± 0.01	2.6 ± 0.9	1.02 ± 0.01	0.9985

Highlights

Saccharomyces cerevisiae biomass is able to form good plasticized films.

The presence of plasticizer reduced the initial degradation temperature of yeast films.

Glycerol decreased Young's modulus and tensile strength, but slightly increased elongation at break.

Hydration was increased with the increasing amounts of glycerol in film formulations.

Characterisation of yeast films evidenced the potentiality for packaging applications.

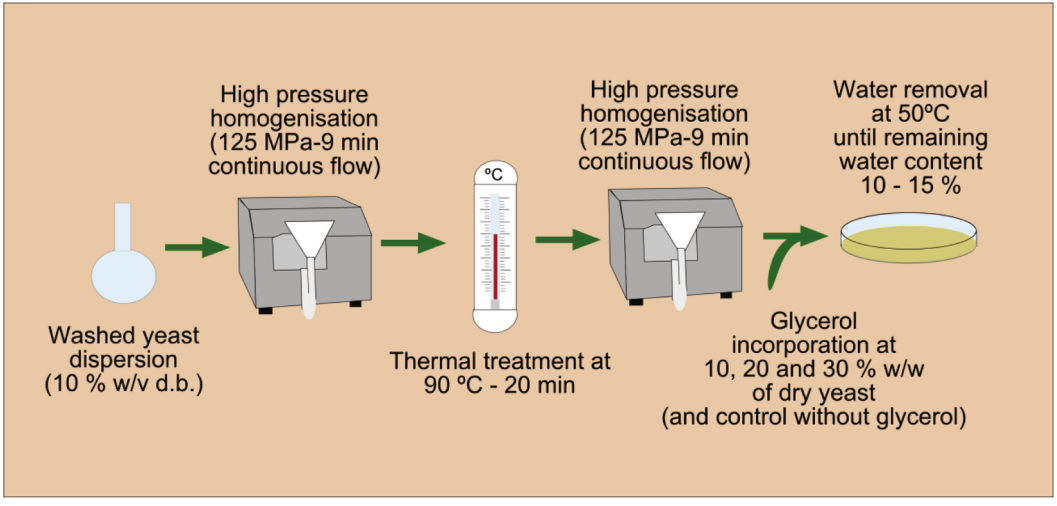
High pressure homogenisation (125 MPa-9 min continuous flow)
ºC
High pressure homogenisation (125 MPa-9 min continuous flow)
Water removal at 50ºC until remaining water content 10 - 15 %
Washed yeast dispersion (10 % w/v d.b.)
Thermal treatment at 90 ºC - 20 min
Glycerol incorporation at 10, 20 and 30 % w/w of dry yeast (and control without glycerol)

Figure 1

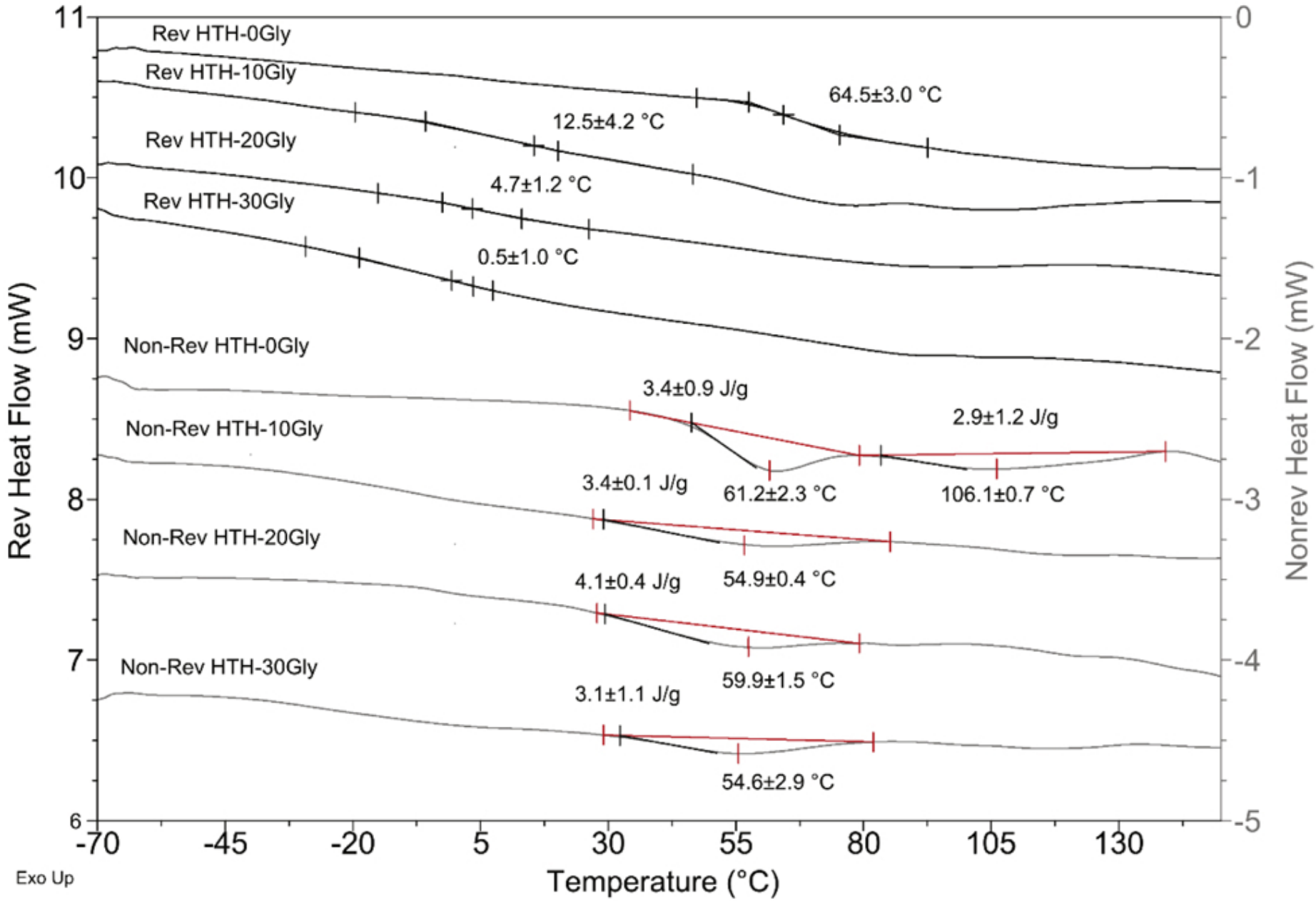
Rev HTH-0Gly
Rev HTH-10Gly
Rev HTH-20Gly
Rev HTH-30Gly
Non-Rev HTH-0Gly
Non-Rev HTH-10Gly
Non-Rev HTH-20Gly
Non-Rev HTH-30Gly
64.5±3.0 °C
12.5±4.2 °C
4.7±1.2 °C
0.5±1.0 °C
3.4±0.9 J/g
2.9±1.2 J/g
61.2±2.3 °C
106.1±0.7 °C
3.4±0.1 J/g
54.9±0.4 °C
4.1±0.4 J/g
59.9±1.5 °C
3.1±1.1 J/g
54.6±2.9 °C
Rev Heat Flow (mW)
Nonrev Heat Flow (mW)
Temperature (°C)
Exo Up
11
10
9
8
7
6
0
-1
-2
-3
-4
-5
-70
-45
-20
5
30
55
80
105
130

Figure 2

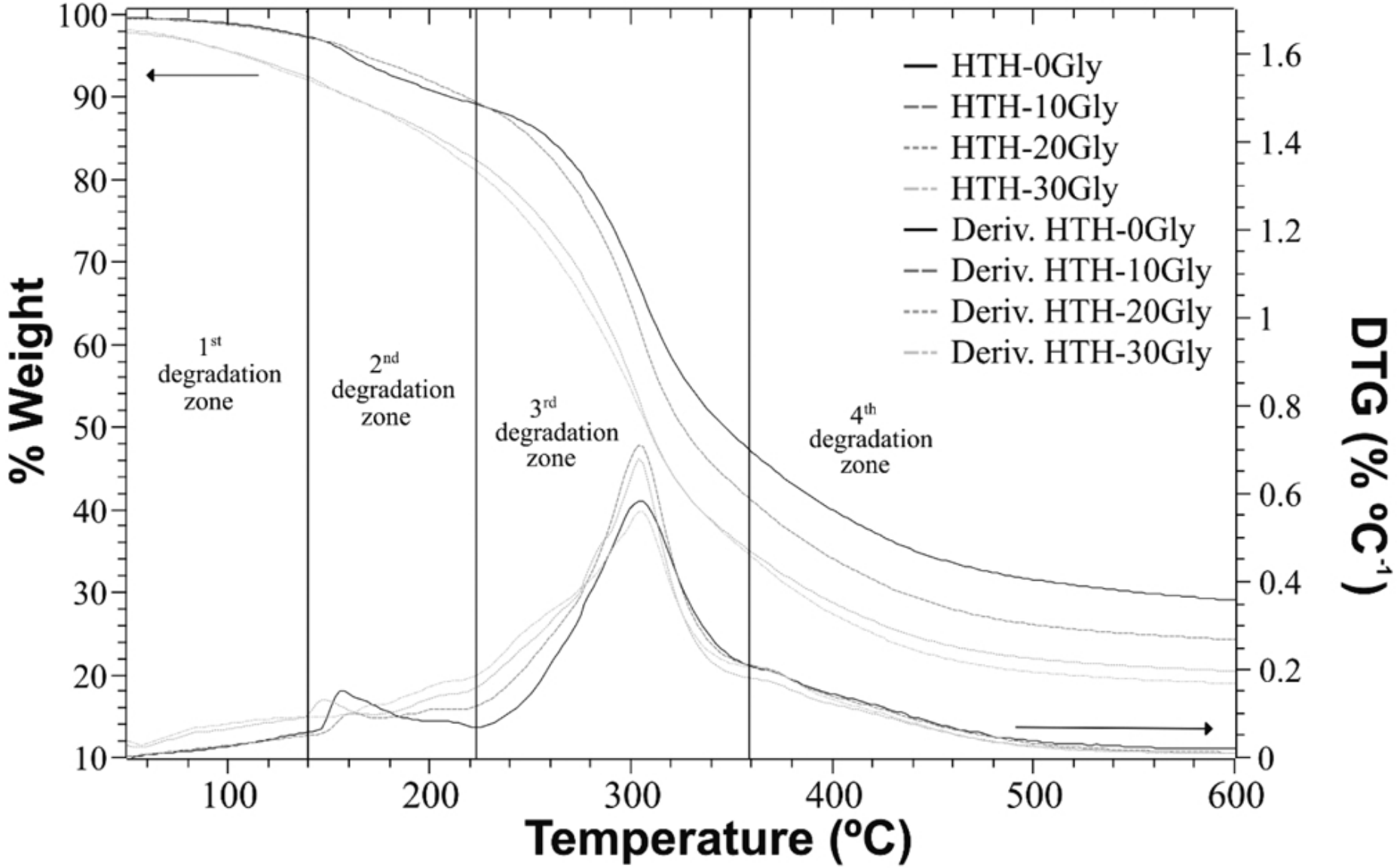
% Weight
DTG (% °C-1)
Temperature (°C)
HTH-0Gly
HTH-10Gly
HTH-20Gly
HTH-30Gly
Deriv. HTH-0Gly
Deriv. HTH-10Gly
Deriv. HTH-20Gly
Deriv. HTH-30Gly
1st degradation zone
2nd degradation zone
3rd degradation zone
4th degradation zone

Figure 3

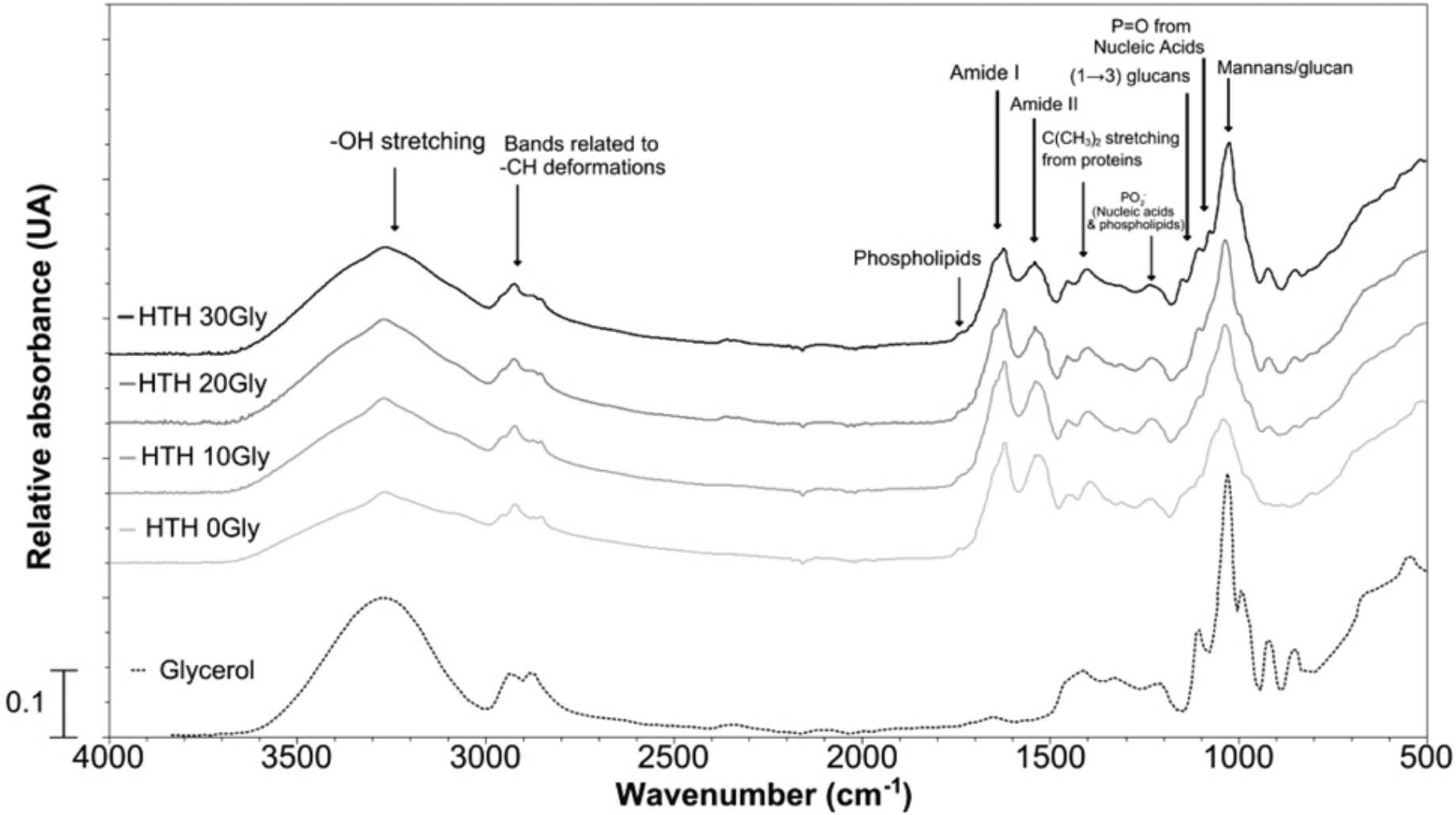
Relative absorbance (UA)
-OH stretching
Bands related to
-CH deformations
Amide I
Amide II
P=O from
Nucleic Acids
(1→3) glucans
Mannans/glucan
C(CH3)2 stretching
from proteins
PO2-
(Nucleic acids
& phospholipids)
Phospholipids
HTH 30Gly
HTH 20Gly
HTH 10Gly
HTH 0Gly
Glycerol
0.1
4000
3500
3000
2500
2000
1500
1000
500
Wavenumber (cm-1)

Figure 4

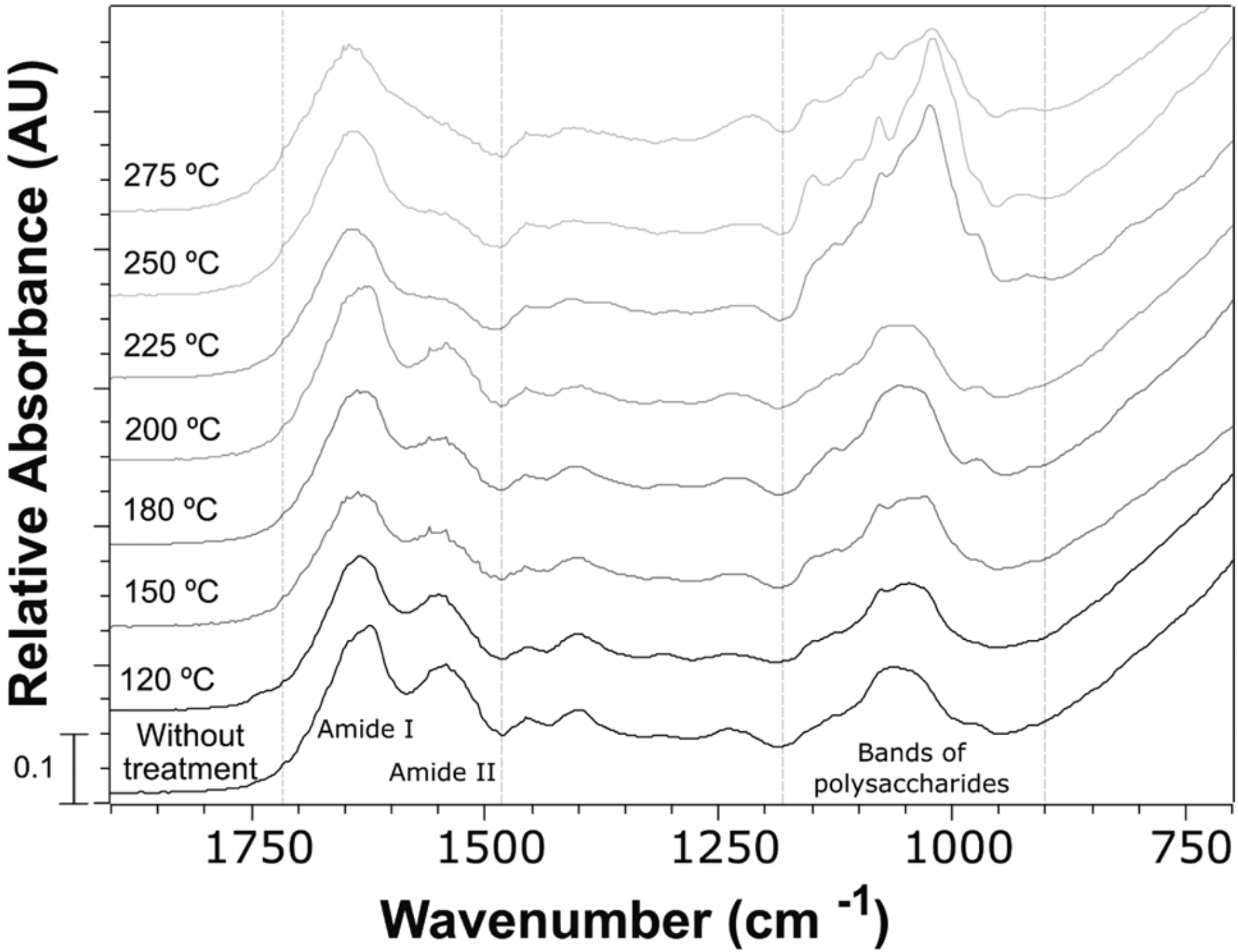
Relative Absorbance (AU)
275 ºC
250 ºC
225 ºC
200 ºC
180 ºC
150 ºC
120 ºC
Without treatment
0.1
Amide I
Amide II
Bands of polysaccharides
1750
1500
1250
1000
750
Wavenumber (cm -1)

Figure 5

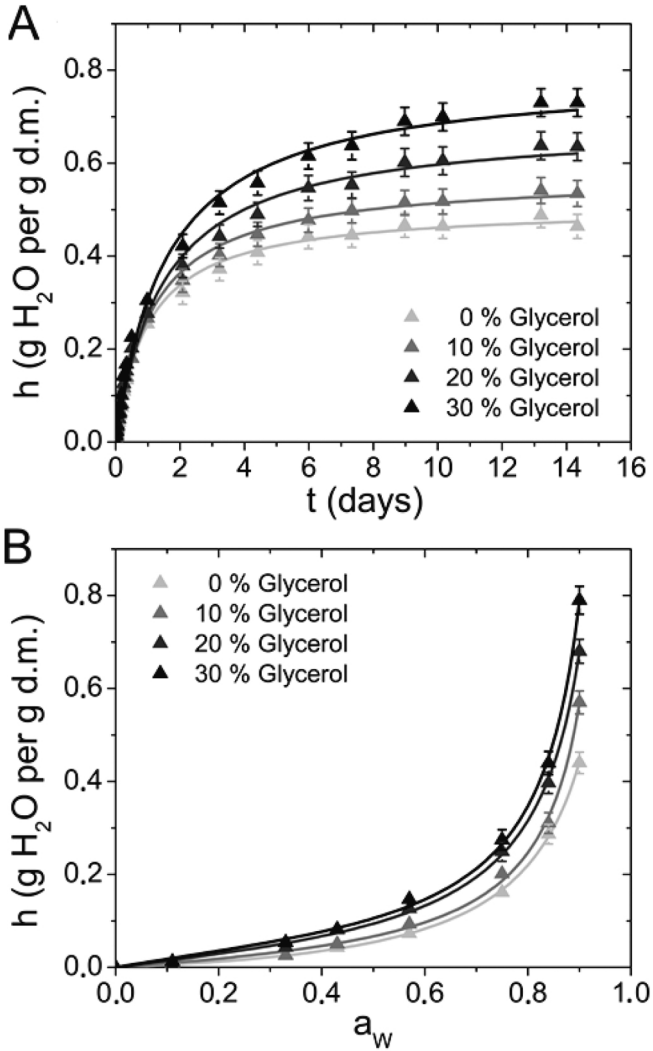
A
h (g H2O per g d.m.)
t (days)
0 % Glycerol
10 % Glycerol
20 % Glycerol
30 % Glycerol
B
h (g H2O per g d.m.)
aw
0 % Glycerol
10 % Glycerol
20 % Glycerol
30 % Glycerol

Figure 6